

CLAIMS

Ado D2

1. A method of loading lipid-like vesicles having a membrane permeable to a chemical species to be loaded from a loading solution wherein the concentration of the thus loaded chemical species within the vesicle is greater than the concentration of the chemical species in the loading solution and the loaded chemical species can be substantially maintained within the vesicle for at least one-quarter hour following loading, comprising:

inducing a pH gradient between the inside and outside of the vesicle membrane while the vesicle is in the loading solution containing the chemical species said pH gradient being selected to drive said chemical species into said vesicles.

2. A method of loading lipid-like vesicles having a membrane permeable to a chemical species to be loaded and for substantially maintaining the loaded chemical species within the vesicle for at least one-quarter hour following loading by inducing a pH gradient across the membrane, comprising:

(1) incorporating within the vesicle a buffer solution buffered to a selected acid or alkaline pH and having a selected molarity and at least one selected pKa approximately equal to the selected buffer pH, the membrane being substantially impermeable to the buffer for at least one-quarter hour following loading of the chemical species;

(2) positioning the vesicles in a bulk solution having a selected pH; and

(3) providing the bulk solution with a chemical species having one or more selected acid pH responsive groups if the buffer is alkaline or one or more basic pH responsive groups if the buffer is acidic wherein the pH of the bulk solution is at least respectively 0.5, 0.3 or 0.2 of a pH unit higher than the pH of the buffer if the buffer is acidic and the chemical species has respectively one,

two, or three or more basic pH responsive groups, or the pH of the bulk solution is at least respectively 0.5, 0.3 or 0.2 of a pH unit lower than the pH of the buffer if the buffer is basic and the chemical species has respectively one, two, or three or more acid pH responsive groups, the pH responsive groups of the chemical species having one or more acid pH responsive groups have a pKa that is generally lower than or equal to the pH of the bulk solution and generally higher than or equal to 3.5 and the pH responsive groups of the chemical species having one or more basic pH responsive groups have a pKa that is generally higher than or equal to the pH of the bulk solution and generally lower than or equal to 11.

3. A method according to Claim 2 wherein the pH responsive group or groups are acid pH responsive groups and the buffer has a pKa in the range of about 10.

4. A method according to Claim 3 wherein the chemical species has a pKa from about 4-7.

5. A method according to Claim 4 wherein the pH responsive group is a carboxyl group.

6. A method according to Claim 2 wherein the pH responsive group or groups are basic pH responsive groups, and the buffer has a pKa in the range of about 5.

7. A method according to Claim 6 wherein the chemical species has a pKa from about 7-10.

8. A method according to Claim 7 wherein the pH responsive group is an amino group.

9. A method according to Claim 8 wherein the chemical species is an amine.

10. A method according to Claim 2 wherein the vesicle is prepared in the buffer and incorporates the buffer via mixing and sonication.

11. A method according to Claim 2 wherein the pH of the bulk solution is from about 7.0 to about 7.8.

12. A method according to Claim 11 wherein the pH of the bulk solution is about 7.4.

13. Vesicles prepared according to Claim 2 wherein the chemical species is a drug.

14. A pharmaceutical preparation for administration in vivo to an animal comprising lipid-like vesicles prepared according to Claim 1 wherein said chemical species is a drug.

15. A pharmaceutical preparation for parenteral administration in vivo to an animal comprising liposomes prepared according to Claim 2 wherein said chemical species is a drug, the osmolarity of the buffer is within the physiological range of the animal, the vesicles are suspended for administration in the bulk solution, and the pH of the bulk solution is physiologically benign.

16. A kit for loading lipid-like vesicles having a membrane permeable to the chemical species to be loaded comprising:

(1) a first compartment having a first solution having membranous lipid-like vesicles incorporating a buffer buffered to a selected acid or basic pH, the buffer having at least one selected pKa approximately equal to the selected buffer pH and a selected molarity and being substantially impermeable to the vesicle for at least one-quarter hour following loading of the chemical species and the first solution having a selected pH such that the

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stability of the vesicle and its buffer can be maintained for a period of at least one week at 4°C.

(2) a second compartment, separate from the first compartment, having a second solution having a selected pH;

(3) a chemical species permeable to the vesicle having a selected pKa and one or more selected acid pH responsive groups if the buffer is basic or one or more basic pH responsive groups if the buffer is acidic, the chemical species being initially present in a selected one of the two solutions with the second solution having a pH such that a mixture of the first and second solutions would have a pH respectively of at least, 0.5, 0.3, or 0.2 of a pH unit higher than the pH of the buffer if the buffer is acidic and the chemical species has respectively one, two, or three or more basic pH responsive groups or a pH at least, respectively, 0.5, 0.3 or 0.2 of a pH unit lower than the pH of the buffer if the buffer is basic and the chemical species has respectively one, two or three or more acid pH responsive groups, the pH responsive groups of the chemical species having one or more acid pH responsive groups have a pKa that is generally lower than or equal to the pH of the mixture of the first and second solution and generally higher than or equal to 3.5 and the pH responsive groups of the chemical species having one or more basic pH responsive groups have a pKa that is generally higher than or equal to the pH of the mixture of the first and second solutions and generally lower than or equal to 11.

17. A kit as set forth in Claim 16 wherein said chemical species is a drug.

18. A kit as set forth in Claim 17 wherein the mixture will have a pH that is physiologically benign in regard to the blood of a mammal.

19. A kit as set forth in Claim 18 further including means for parenterally delivering the mixture to a mammal in vivo.

20. A kit for loading lipid-like vesicles having a membrane permeable to an acid or basic chemical species to be loaded comprising:

(1) a first compartment having a first solution having membranous lipid vesicles incorporating a buffer buffered to a selected basic pH if the chemical species to be loaded is an acid or acid pH if the species is a base, the buffer having a selected pK_a and a selected molarity, the membrane being substantially impermeable to the buffer for at least one-quarter hour following loading of the chemical species, the first solution having a selected pH such that the stability of the vesicle and its buffer will be maintained for a period of at least one week at 4°C;

(2) a second separate compartment having a first substance which when combined with the first solution will adjust the pH of the first solution so as to provide a predetermined pH gradient between the buffer within the vesicle and the pH adjusted first solution; and

(3) a third separate compartment having a second substance which when combined with the pH adjusted first solution will further change the pH of said solution to a physiologically benign value with regard to the blood of a mammal.

21. A kit as set forth in Claim 20 further including a selected chemical species.

22. A kit as set forth in Claim 21 wherein the selected chemical species is a drug.

23. A kit as set forth in Claim 22 further including a means for parentally delivering the vesicle solution

having the physiologically benign adjusted pH to a mammal in vivo.

24. A method of detoxifying an animal suffering from an overdose of a chemical species with basic pH responsive groups comprising injecting the animal with a solution having a physiologically benign pH with respect to the animal, the solution having large volumes of liposomes having a buffer solution buffered to a pH generally lower than or equal to 5.4 and the buffer having at least one selected pKa and a selected molarity within the physiological range of the animal the liposomes being substantially impermeable to the buffer for at least one hour after injection.

25. A method for detoxifying an animal suffering from an overdose of a chemical species with acid pH responsive groups the chemical species being permeable to liposomes comprising: injecting the animal with a solution having a physiologically benign pH with respect to the animal, the solution having large volumes of liposomes having a buffer solution buffered to a pH generally higher than or equal to 9.4 and having a selected molarity and selected pKa, the liposomes being substantially impermeable to the buffer for at least one hour after injection.

26. A method of loading lipid-like vesicles having a membrane permeable to a chemical species to be loaded and substantially maintaining the loaded chemical species by inducing a pH gradient across the membrane within the vesicle for at least one-quarter hour following loading, comprising:

(1) incorporating within the vesicle a buffer solution buffered to a selected acid or alkaline pH and having a selected molarity and at least one selected pKa, the membrane being substantially impermeable to the buffer

for at least one-quarter hour following loading of the chemical species;

(2) positioning the vesicles in a bulk solution having a selected pH of either 0.5 to 3 pH units lower or pH units higher than the pH of the buffer thereby establishing a transmembrane electrical potential and a positive charge inside the vesicle if the pH outside the vesicle is more acid than inside or a negative charge inside the cell if the pH outside the cell is more basic than inside;

(3) providing in the bulk solution a chemical species having hydrophobic negatively-charged ions if the membrane charge within the vesicle is positive or hydrophobic positively charged ions if the membrane charge within the vesicle is negative.

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